METHOD OF PRODUCING VEGETABLE PUREE

FIELD OF INVENTION

This invention relates to a method of producing vegetable puree.

BACKGROUND OF THE INVENTION

Vegetable puree products, such as carrot puree, are marketed as independent products or used as semi-finished products in various branches of the food industry. A defined amount of puree is frequently added to vegetable juices in order to produce high quality full bodied products.

A conventional enzymatic vegetables puree process suitable for e.g. carrots may comprise washing, sorting, peeling, coarsely crushing by means of a hammer mill, blanching by heated to 80-85°C, and enzymatic treatment with macerating enzymes before the vegetable mash is separated into pomace and liquid (serum) using e.g. a sieve, a decanter or a centrifuge. Using a suitable evaporator, typically with aroma recovery, the serum may be thickened before being remashed back into the decanter pomace which is then used as puree. The separation step of a conventional process may cause large amounts of vegetable solids to be lost.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide improved methods of producing vegetable purees wherein the separation step may be omitted. Accordingly, the invention provides in a first aspect a method of producing a vegetable puree, comprising the sequential steps of: a) crushing or chopping the vegetable into pieces of 1 to 30 mm; b) blanching the vegetable pieces at a temperature of 60 to 90°C; c) contacting the blanched vegetable pieces with a macerating enzyme activity; and d) blending the macerated vegetable pieces and obtaining a puree.

With this invention the mechanical and thermal treatments can – by selecting the right enzymes - be reduced to a minimum. The process of the invention allows close to 100% yield as the rather large yield losses normally experienced in the separation step are eliminated. The milling operation can be optimized securing less energy consumption and less damage to the cells. The pasteurization can also be performed in the enzymation tank or in a heat exchanger directly connected with the tank. Further it minimizes damages to the cells resulting in a very smooth consistency of the resulting suspension, high viscosity, better storage stability, improved organoleptic/sensoric properties, and, when used in juice production, in increased cloud stability. The high viscosity is very important when the puree is applied as baby food or as an ingredient in juices, soups or sauces, as it enhances mouthfeel and reduces the need for additional thickening agent. Furthermore, as the cells are intact valuable constituents are protected from

oxidation thereby increasing the nutritional value of the final product.

High amounts of soluble saccharides are released when using a pectinase product. Without being bound by theory it is believed that the difference in viscosity observed between the monocomponent polygalacturonase and the multi component enzyme composition may be due to the multi component enzyme composition further degrading the polysaccharides into oligo- and monosaccharides, while the monocomponent polygalacturonase just releases the polysaccharides with practically no further degradation.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 shows results of high performance size exclusion chromatography. Curve A is Negative control, curve B is PG 1 and curve C is Pectinex.

DETAILED DISCLOSURE OF THE INVENTION

In the context of this invention a vegetable puree is a liquid composition comprising vegetable dry substances. The dry substances may be present as a suspension of vegetable solids comprising fully homogenized vegetable material, intact single cells, clusters of several cells, or a mixture of these. The puree of the invention may be used in any kind of food stuff, e.g. in baby foods, fruit juices, ketchups, soups or sauces.

The puree of the invention may be produced from one vegetable or from a number of different vegetables selected from the list comprising root vegetables such as carrots celeries, beetroots, radishes, horse-radishes; fruit vegetables such as apples, pears, grapes, tomatoes, citrus (orange, lemon, lime, mandarin), prunes, cherries, peas, beans, tomatoes, paprikas, cucumbers, and pumpkins; leaf and flower vegetables such as spinach, cabbage, and cauliflower. According to this invention especially puree from carrot, *Daucus carota* var. *sativa*, is preferred. Any suitable cultivar types of carrot is may be used including but not limited to the carrot cultivars *Parisian market, Oxheart, Amsterdam forcing, Chantenay, Nantes, Danvers, Imperator, Flakkee, Berlikum*, and *Kuroda*. For further types of carrots or other vegetable suitable for the present invention, please refer to World Vegetables. Principles, Production, and Nutritive values. Second Edition. Vincent E. Rubatzky and Mas Yamaguchi (Ed.), 1997, pp 418-456, Chapman & Hall.

The vegetable is crushed in a mill, or chopped or sliced with knives into pieces of 1 to 30 mm. One important effect of the process step (a) is to increase the surfaces area and thus allowing better access for the enzymes.

The term “blanching” means a short lasting subjection of vegetable pieces to the action of hot water or steam. One important effect of the thermal treatment is to stop unwanted enzymatic action, another to weakening the tissues and thus allowing better access

for the enzymes. The blanching step (b) may have a duration of 10 sec to 15 minutes, preferably from 30 sec to 10 minutes, more preferably from 1 to 8 minutes, and most preferably from 2 to 6 minutes. The blanching step (b) may have a temperature within the range of 45-120°C, preferably within the range of 50-110°C, more preferably within the range of 60-100°C, and even more preferably within the range of 70-90°C.

Suitable macerating enzyme compositions for the invention comprise pectin depolymerases, such as polygalacturonase (EC 3.2.1.15), pectin lyase (EC 4.2.2.10), a rhamnogalacturonan acetyl esterase (EC 3.1.1.6) and pectate lyase (EC 4.2.2.2). The enzyme composition is preferably low in pectin methylesterase. The macerating enzymes weaken the intercellular cementing material of plant tissue called the middle lamella. The main component of the middle lamella is insoluble protopectin, which, however, becomes soluble after restricted degradation.

The enzyme compositions applied may be multi component enzyme compositions, such as the composition Pectinex Ultra SP-L from Novozymes A/S and derived from *Aspergillus aculeatus*, high in polygalacturonase activity, or a mono component pectin lyase such as the Pectinex Smash XXL also from Novozymes A/S or a mono component enzyme having polygalacturonase activity such as a polygalacturonase cloned from *Aspergillus aculeatus* CBS 101.43, as described in US 6,159,718 and available from Novozymes A/S or having pectate lyase activity, such as a pectate lyase derived from *Bacillus licheniformis*, or having pectin lyase activity, such as a pectin lyase derived from *Aspergillus*, preferably *A. niger, A. aculeatus,* or *A.oryzae* or having rhamnogalacturonan acetyl esterase activity such as the monocomponent rhamnogalacturonan acetyl esterase from *A. aculeatus* and described in US 5,585,256 A1.

Macerating enzymes are used in effective amounts, preferably within the range of 0.05 to 5000g enzyme protein/ton vegetable, more preferably within the range of 0.5 to 500g enzyme protein/ton vegetable, even more preferably within the range of 1-250g enzyme protein/ton vegetable and most preferably within the range of 5-100 g enzyme protein/ton vegetables. Preferably the duration of step (c) is within the range of 2 minutes to 24 hours, such as within the range of 5 minutes to 5 hours, such as within the range of 15 minutes to 4 hours, such as within the range of 30 minutes to 2 hours. Preferably the temperature is within the range of 10 to 120°C, preferably within 40-70°C.

The term “blending” means a gentle homogenization, just strong enough to disrupt the enzymatically weakened vegetable tissue to produce a suspension of single cells without disrupting the individual cells. The blending step (d) may be performed using a blender with rotating knives at a speed of 100-50000 rpm, or more preferably of 1000-10000 rpm. The blending may be performed for a period of 10 sec to 10 hours, preferably from 15 sec to 5 hours,

more preferably from 20 sec to 1 hour, even more preferably from 25 sec to 30 minutes, such as from 30 sec to 5 minutes.

In a preferred embodiment wherein the vegetable is carrot, the pH is preferably adjusted to within the range of 3 to 5, such as around 4.0, using a suitable acidifying agent, e.g. citric acid, lemon juice, lactic acid, tartaric acid, malic acid, phosphoric acid, or acetic acid, and preferably the enzyme applied is a polygalacturonase.

Following the enzyme treatment in step (c) or the blending step (d) remaining enzyme activity is preferably inactivated, e.g. by heating the mixture to a temperature of 50-120°C, or preferably 60-90°C, the precise temperature chosen according to the thermal stability of the specific enzyme composition used.

The invention is further illustrated in the following example, which is not intended to be in any way limiting to the scope of the invention as claimed.

EXAMPLES

Materials and methods

Enzymes

A polygalacturonase I (PG I) cloned from *Aspergillus aculeatus*, as described in US 6,159,718.

The commercial enzyme preparation Pectinex Ultra SP-L which is derived from *Aspergillus aculeatus.* Pectinex Ultra SP-L derived from *Aspergillus aculeatus* is a multi component enzyme preparation comprising pectolytic and a range of hemicellulolytic activities. Pectinex Ultra SP-L with an activity of 26,000 PG/ml (pH 3.5) is available from Novozymes A/S.

The commercial enzyme preparation Pectinex 100L Plus, derived from *Aspergillus niger* is a multicomponent polygalacturonase and pectin lyase preparation free of pectin esterase. Pectinex 100 L Plus with a standard activity of 5,000 UPTE/ml (pH 3.5) is available from Novozymes A/S.

A rhamnogalacturonan acetyl esterase preparation from *A. aculeatus* as described in US 5,585,256 A1.

Determination of polygalacturonase activity (PG)

The standard activity is determined by the measurement of the viscosity reduction of a solution of pectic acid at pH 3.5 and 20°C (68°F). See the Analytical Method for SM-1030.02/02 available from Novozymes A/S for further information.

Determination of pectin transeliminase activity (UPTE)

The method is based on the enzyme's degradation of dissolved pectin, which is measured using a spectrophotometer (e.g. Hitachi 1100). One pectin transeliminase unit (UPTE) is defined as the amount of enzyme which raises absorbance by 0.01 absorbance units per minute under standard conditions: Substrate concentration 0.5% Obipectin, 30°C, pH 5.4, Reaction time 10 minutes and absorbance at 238 nm.

A citrate-phosphate buffer and a pectin substrate are used for the analysis.

Citric acid buffer 0.1 M is prepared from 21.0 g citric acid ($C_6H_8O_7$,H_2O), e.g. Merck 244 and demineralized water up to 1000 mL. Dipotassium hydrogen phosphate buffer 0.1 M is prepared from 17.4 g K_2HPO_4 dipotassium hydrogen phosphate, e.g. Merck 5104and demineralized water up to 1000 mL. A citrate-phosphate buffer is prepared from 290 mL 0.1 M citric acid buffer and 710 mL 0.1 M dipotassium hydrogen phosphate.

The pectin substrate is prepared from 0.50 g Obipectin (Braunband), 0.5 mL 96% ethanol and 50 mL 0.1 M citrate-phosphate buffer which are heated to the boiling point and stirring for 15 minutes. The solution is cooled to room temperature and the pH adjusted to 5.4 using 1 M NaOH and demineralized water is added up to 100 mL.

One blind is prepared: 2.5 mL substrate is pipetted into a 10 mL test tube. The tube is heated in 30°C water bath for approx. 5 minutes. 0.5 mL demineralized water is added and the tube shaken. Absorbance is measured at 238 nm on a spectrophotometer and the blind set at zero.

The enzyme samples are diluted with demineralized water to approx. 7 UPTE/mL. 2.5 mL substrate is pipetted into a 10 mL test tube. The tube is heated in a 30°C water bath for approx. 5 minutes. 0.5 mL enzyme solution is added and the tube shaken. Absorbance is measured after exactly 10 minutes.

The pectin lyase activity is calculating using the formula;

$$\text{Activity (UPTE / g)} = \frac{\Delta \text{Abs} \cdot V_1}{T \cdot 0.01 \cdot M \cdot V_2 \cdot 1.11}$$

wherein ΔAbs = Abs_{238nm}(10 mins) - Abs_{238nm}(0 mins), V_1 = Flask volume, T = 10 mins, M = Weight of sample in g, V_2 = Enzyme volume, 0.01 = Increase in absorbance.

A detailed description of the analytical method (EB-SM-0368.02/01) is available on request from Novozymes A/S.

Various methods

Dry substance (DS) was determined after 24 hours incubation at 105°C.

Determination of cloud stability of diluted puree was made by mixing 5 ml puree with 5

ml de-ionized water. The amount of formed sediment was measured after 24 hours incubation at 5°C. A high amount of sediment after storage corresponds to juice with good cloud stability.

The release and molecular weight distribution of the soluble polysaccharides, oligosaccharides and monosaccharides was followed using high performance size exclusion chromatography. The samples were filtered through a 0.46 micromillimeter filter and separated on four Guardcolumn TSK-gel PWXL Toso Haas; G2500; G3000; G4000; G5000 connected in a row (0.4 M acetic acid adjusted to pH 3.0 using sodium acetate as eluent). Detection of eluted saccharides was performed using a refractive index detector RID6A (Shimadzu Japan).

Viscosity was determined by placing 50 ml puree in a Rapid Visco Analyser RVA-4 (Newport Scientific, Australia) at 25°C using a shear of 200 rpm. The viscosity was recorded over 2 minutes and the mean result was calculated in centiPoise (cP).

Free color of carrot purees was determined as *heptane extractable carotenoides.* 1 mL juice was pipetted into a centrifuge vial. 2 mL heptane was added and the free carotenoides were extracted by turning and mixing with caution. The heptane phase (top) was transferred to a new vial. Extraction was preformed twice and the heptane phases were pooled. Carotenoide content was measured by spectrophotometric scan at 476 nm with heptane as reference.

Total color of carrot purees may be determined as *propanol extractable carotenoides.* 1 mL juice was weighed into a centrifuge vial. 12 mL propan-2-ol was added and the vial was mixed and left for sedimentation over night. The sample was centrifugation for 2 minutes at 3000 rpm and the supernatant was transferred to a new vial. The extraction procedure was repeated with 5 ml until the cells were colorless. Supernatants were pooled and centrifuged for 5 minutes at 4000 rpm. Spectrophotometric measurement was performed at 476 nm with propan-2-ol as reference.

The number of undamaged vegetable cells compared to the total number of released cells may be estimated from the ratio between free color of carotenoides and total color. Microcopical examination may also give a good estimate of the amount of cells damaged.

Example 1

Carrots were purchased from the local market. The dry substance of the carrots was 11.75%. 1 kg of the carrots was peeled by hand and cut into 1 mm thick slices. The slices were blanched in 4 l of de-ionized water for 3 minutes at 80°C. The water was drained and the carrot slices drip dried for about 10 minutes. 100 ml 100 mM sodium citrate buffer pH 4 was added to 100 g of blanched carrots (dry substance 6.85%). The temperature was

equilibrated in a water bath at 45°C. Enzyme or water was added and gently stirred. The enzyme compositions PG I and Pectinex Ultra SP-L were dosed as 50 g enzym protein/ton carrots and 12.5 g enzym protein/ton carrots, respectively. The mixture was incubated for 2 hours at 45°C without further stirring.

The enzymes were inactivated by heating the mixture to 80°C and holding the temperature for 10 minutes. Blended was performed for 1 minute in a Waring 1.2 l heavy duty laboratory seven speed blender 8012G with rotating knifes, and set at speed 2 corresponding to 7000 rpm.

The formed carrot puree was filtered through a 0.5 mm screen. Dry substance weight of particles larger than 0.5 mm (residual) were determined and the puree yield was calculated using the formula: $(\%DS_{blanched\ carrot}-\%DS_{residual})/(\%\ DS_{blanched\ carrot})*100$.

Table 1. The yield, the viscosity, the stability of puree dilutions, and color determined as described above.

Enzyme	Blending	Yield (%)	Viscosity (cP)	Sediment of puree dilutions (ml)	Free/total colour (%)
Negative control	No blending	22.1	18	0	7.73
PG I		26.0	21	0.5	5.12
Pectinex Ultra		37.6	10	0.5	4.67
Negative control	Blending	50.8	73	4	7.34
PG I		99.5	692	9	0.20
Pectinex Ultra		99.5	109	9	0.79

PG I and Pectinex Ultra SP-L represent a cloned monocomponent and a multicomponent pectinase composition, respectively. When the blending step was omitted only a moderate yield was obtained using PG I and Pectinex Ultra SP-L. However, it was observed that by combining blending with an enzyme treatment close to 100% yield was obtained.

Blending affects the viscosity of the produced purees. Without blending the viscosity of the purees ranges from 10-21 cP. Blending increases the viscosity and a synergistic effect was observed between enzyme and blending. In particular, the effect on viscosity of the cloned PG I was unique and resulted in a highly viscous puree (about 700 cP).

By the combined enzyme treatment and blending it was possible to obtain a 100% yield with almost no cell damage. The amount of total cell disruption was less than 1% for both types of enzyme products.

Fig. 1 shows results of high performance size exclusion chromatography. Curve A is negative control, curve B is PG 1 and curve C is Pectinex. Mw is milli volt, - a reaction is

proportional to the mass of molecules. Large molecules are eluted first, small molecules later. The figure indicates that the multi component enzyme composition (curve C) further degrades the released polysaccharides into oligo- and monosaccharides, while the monocomponent polygalacturonase (curve C) just releases the polysaccharides with practically no further degradation.

Example 2

Carrots were purchased from the local market and processed as described in Example 1 except that the enzyme used was Pectinex 100L Plus. The enzyme preparation was dosed in the range of 0.05 – 0.4 ml/kg carrots and compared to a negative control.

In table 2 the data for yield and viscosity of the resultant puree is shown. The data show that a puree yield of more than 90% is possible and that the enzyme action can be controlled so that more pectin is solubilized and relatively less is degraded resulting in a high viscosity.

Table 2. Effect of Pectinex 100L Plus dosage on yield and viscosity of carrot puree

Dosage, ml/kg carrots	Puree yield, %	Puree viscosity, cP
0	36.3	38
0.05	79.4	61
0.1	88.1	222
0.2	90.2	333
0.4	93.0	396

Example 3

Golden Delicious apples were purchased from the local market. The dry substance of the carrots was 9.8% and the pH 3.7. The apples were peeled by hand and cut into 2 mm thick slices. One part of apple slices were blanched in one part of de-ionized water for 5 minutes at 80°C. The blanched apple slices were incubated for 1 hours at 45°C in an enzyme solution comprising Pectinex Ultra SP-L (dosage range 0,1-0,4 ml/kg apple).

The enzymes were inactivated by heating the solution to 80°C and holding the temperature for 10 minutes. Blending was performed for 1/2 minute in a Waring 1.2 l heavy duty laboratory seven speed blender 8012G with rotating knifes, and set at speed 1 corresponding to 3500 rpm.

The formed apple puree was filtered through a 0.5 mm screen. Dry substance weight of particles larger than 0.5 mm (residual) were determined and the puree yield was calculated using the formula: $(\%DS_{\text{blanched carrot}}-\%DS_{\text{residual}})/(\%\ DS_{\text{blanched carrot}})*100$.

The yield as a function of enzyme dosage is shown in table 3 showing that a yield of more than 90% is possible.

As in the previous examples blending affected the viscosity of the produced purees. Without blending the viscosity of the purees was less than 20 cP. Blending increased the viscosity and a synergistic effect was observed between enzyme dosage and blending.

Microscopical examination showed that the puree consisted of a suspension of single intact cells. Autoclaving at 121°C for 12 minutes did not affect the cell structure.

Table 3. Effect of Pectinex Ultra SP-L dosage on yield and viscosity of apple puree

Dosage, ml/kg apples	Puree yield, %	Puree viscosity, cP
0	56,4	27
0.1	76,8	58
0.2	86,1	76
0.3	85,7	86
0.4	91,9	82

Example 4

Pears were purchased from the local market and processed as described for apples in Example 3. The pears had a pH of 3.8.

As shown in table 4 a yield of more than 80% was achieved and an even higher yield is obtainable by increasing the enzyme dosage.

Like with carrots blending affected the viscosity of the produced purees. Without blending the viscosity of the purees was less than 20 cP. Blending increased the viscosity and a synergistic effect was observed between enzyme dosage and blending.

Table 4. Effect of Pectinex Ultra SP-L dosage on yield and viscosity of pear puree

Dosage, ml/kg pears	Puree yield, %	Puree viscosity, cP
0	35.6	28
0.05	38.6	31
0.1	66.0	75
0.2	73.4	100
0.5	83.1	100

Example 5

Carrots were purchased from the local market and processed as described in

Example 1 except that the enzyme used was rhamnogalacturonan acetyl esterase. Rhamnogalacturonan acetyl esterase was added corresponding to 25 g enzyme protein/ton of blanched carrot. The carrot slices were incubated for 2 hours at 45°C, pH 6. The enzymes were inactivated by heating the mixture to 80°C and holding the temperature for 10 minutes. The mixture was blended for 1 minute at 7000 rpm. The effect on viscosity and yield is listed in table 5.

Table 5. Effect of polygalacturonase on yield and viscosity of carrot puree

Treatment	Viscosity (cP)	Yield (%)
Blank – no enzyme	174	78.4
Rhamnogalacturonan acetyl esterase	740	97.8

Surprisingly it was seen that modification of the rhamnogalacturonan polymer induces maceration of the pectic plant cell wall and the middle lamella. The high viscosity originates from solubilization of the polysaccharide fraction: